PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT25622	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/IT2004/000287	International filing date *(day/month/year)* 19.05.2004	Priority date *(day/month/year)* 19.05.2003
International Patent Classification (IPC) or national classification and IPC C12N15/82, C07K14/01		
Applicant ENEA-ENTE PER LE NUOVE TECNOLOGIE, L'ENERG.. et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18.03.2005	17.08.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Mundel, C Telephone No. +49 89 2399-7314

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2004/000287

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☒ This report is based on translations from the original language into the following language English, which is the language of a translation furnished for the purposes of:

   ☒ international search (under Rules 12.3 and 23.1(b))
   ☐ publication of the international application (under Rule 12.4)
   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-36 as originally filed

Sequence listings part of the description, Pages

1-10 as originally filed

Claims, Numbers

1-25 received on 30.03.2005 with letter of 18.03.2005

Drawings, Sheets

1/16-16/16 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2004/000287

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-25
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-25
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No. PCT/IT2004/000287

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to a mutated V1/AR1/AV1 or C1/AL1/AC1 gene sequence of a tomato infecting geminivirus wherein the mutations consist of point mutations distributed along the sequence in such a way that the continuous homology between the mutated sequence and the corresponding viral gene sequence is below or equal to 8 nucleotides, said mutated sequence encoding for a capsid protein or for a Rep protein, to synthetic constructs comprising such a mutated gene sequence, to expression vectors comprising such constructs, to transgenic plants and seeds comprising such mutated gene sequence and to methods for the preparation of transgenic plants, plant tissues or cells thereof having long lasting resistance against geminiviruses.

2. New claims 1-25 filed with the letter of 17.03.2005 seem to comply with the requirements of Article 19(2) and 34(2)(b) PCT.

   The remarks filed by the Applicant in the letter of 17.03.2005 have been taken into account for drafting the International Preliminary Examination Report (IPER).

3. Novelty and inventive step; Article 33(2) and 33(3) PCT.

   In transgenic plants resistant to geminiviruses due to the expression of a viral protein, loss of resistance is observed after a certain time. None of the documents cited in the International Search Report (ISR) discloses or even suggests that the loss of resistance observed in the transgenic plants could be due to geminivirus-mediated silencing of the transgene. Therefore, the skilled person would not have been motivated to modify the sequence of the transgenes according to the present application.

   Thus, claims 1-25 are to be considered as novel (Article 33(2) PCT) and inventive (Article 33(3) PCT).

Re Item VIII
Certain observations on the international application

1. Claim 1 refer to a mutated V1/AR1/AV1 or C1/AL1/AC1 gene sequence of a tomato infecting geminivirus encoding for a capsid protein or for a Rep protein. It is not clear if this protein is the native geminivirus protein or a modified protein and if the mutated gene encodes the same protein as the non-mutated gene or not. This renders the scope of claim 1 unclear.

2. In claim 3, there is no limit (minimal or maximal) to the size of the truncation what renders the scope of the claim unclear.

3. The wording of claim 5 is confuse. It is not clear if claim 5 is restricted to the sequences disclosed in SEQ ID NO: 3 and SEQ ID NO: 5 or if these sequences are only given as examples.

   This remark also applies mutatis mutandis to claim 7.

4. Claim 12 b) refers to the mutagenesis of the viral sequence so as to make it an ineffective target of the post-transcriptional gene silencing induced by the infecting geminivirus. The attention of the Applicant is drawn that only two types of mutagenesis have been disclosed in the present application : (1) point mutations distributed along the sequence in such a way that the continuous homology between the mutated sequence and the corresponding viral gene sequence is below or equal to 8 nucleotides and (2) deletions of the 5' or 3' regions of the viral gene sequence of step a) until the identification of the region of said gene sequence that is an ineffective target of post-transcriptional gene silencing. Only these two types of mutations could be considered as fully supported by the description of the present application (Article 5 PCT when read in combination with Article 6 PCT).

5. In claims 15-17, it is not clear what is meant by "isolate thereof".

CLAIMS

1. Mutated V1/AR1/AV1 or C1/AL1/AC1 gene sequence of a tomato infecting geminivirus wherein the mutations consist of point mutations distributed along the sequence in such a way that the continuous homology between the mutated sequence and the corresponding viral gene sequence is below or equal to 8 nucleotides, preferably below or equal to 5 nucleotides, said mutated sequence encoding for a capsid protein or for a Rep protein, respectively.

2. Mutated V1/AR1/AV1 gene sequence according to claim 1, encoding for a capsid protein having sequence SEQ ID No 7.

3. Mutated C1/AL1/AC1 gene sequence according to claim 1, wherein the mutation further comprises a truncation occurring at 3' terminal so that the mutated sequence encodes for a truncated Rep protein.

4. Mutated C1/AL1/AC1 gene sequences according to claim 3, wherein the truncated Rep proteins consist of 130 aminoacids (Rep 130) to 210 aminoacids (Rep 210).

5. Mutated C1/AL1/AC1 gene sequence according to any of the claims 3 and 4 encoding for Rep 210 SEQ ID No 3 or SEQ ID No 5.

6. Mutated C1/AL1/AC1 gene sequence encoding for Rep 130 SEQ ID No 9.

7. Mutated gene sequence according to any of the claims 1-6 wherein the tomato infecting geminivirus is TYLCSV.

8. Synthetic construct comprising an heterologous polynucleotide sequence containing in the 5'-3' direction:

a) polynucleotide sequence acting as promoter in said plant or tissue or transformed cells;

b) a non translated polynucleotide sequence positioned 5' of the encoding region;

c) a mutated gene sequence according to any of the claims 1 to 7;

d) a sequence acting as transcription terminator, positioned 3' with respect to the mutated gene sequence.

9. Expression vector comprising the construct as defined according to claim 8.

10. Transgenic plant, tissue or plant cells thereof, comprising in their genome a mutated gene sequence according to any of the claims 1 to 7.

11. Seed comprising in its genome a mutated gene sequence according to any of the claims 1 to 7.

12. Method for the preparation of transgenic plants, plant tissue or cells thereof having long lasting resistance against geminiviruses, including the following steps:

a) identification or selection of a viral gene sequence encoding an aminoacid sequence able to confer resistance against geminiviruses;

b) mutagenesis of the viral gene sequence so as to make it an ineffective target of the post-transcriptional gene silencing induced by the infecting geminivirus;

c) insertion of the geminivirus gene sequence mutated in the step b) in the plant, plant tissue or cell thereof, using a construct comprising an heterologous polynucleotide sequence containing in the 5'-3' direction:

i) a polynucleotide sequence acting as promoter in said plant or tissue or transformed cells;

ii) a non translated polynucleotide sequence positioned 5' of the encoding region;

iii) a polynucleotide sequence encoding a geminivirus-derived aminoacid sequence, properly mutagenised to be an ineffective target of the post-transcriptional gene silencing induced by the infecting geminivirus;

iv) a sequence acting as transcription terminator positioned 3' with respect to said polynucleotide sequence

13. Method according to claim 12 wherein the mutations consist of point mutations distributed along the sequence in such a way that the continuous homology between the mutated sequence and the corresponding viral gene sequence is below or equal to 8 nucleotides, preferably below or equal to 5 nucleotides.

14.. Method according to claim 12, wherein the mutagenesis in step b) consists of deletions of the 5' or 3' regions of the viral gene sequence of step a) until the identification of the minimum region of said gene sequence that is an ineffective target of the post-transcriptional gene silencing induced by the infecting geminivirus compare to the original viral

sequence and that said truncated protein maintains the ability to confer resistance against geminiviruses.

15. Method according to any of the claims 12-14 wherein the geminiviruses are selected from the group consisting of species of Mastrevirus, Curtovirus, Begomovirus and Topocuvirus and isolates thereof.

16. Method according to claim 15, wherein Begomoviruses species are selected from the group consisting of TYLCCNV, TYLCGV, TYLCMalV, TYLCSV, TYLCTHV, TYLCV, ACMV, BGMV, CaLCuV, ToCMoV, TGMV, ToGMoV, ToMHV, ToMoTV, ToMoV, ToRMV, ToSLCV, ToSRV, Cotton leaf curl (CLCrV, CLCuAV, ClCuGV, CLCuKV, CLCuMV, CLCuRV), East African cassava mosaic (EACMCV, EACMMV, EACMV, EACMZV), Potato yellow mosaic (PYMPV, PYMTV, PYMV), Squash leaf curl (SLCCNV, SLCV, SLCYV), Sweet potato leaf curl (SPLCGV, SPLCV), Tobacco leaf curl (TbLCJV, TbLCKoV, TbLCYNV, TbLCZV), Tomato leaf curl (ToLCBV, ToLCBDV, ToLCGV, ToLCKV, ToLCLV, ToLCMV, ToLCNDV, ToLCSLV, ToLCTWV, ToLCVV, ToLCV) and isolates thereof.

17. Method according to claim 15, wherein the species belonging to the genus Mastrevirus, Curtovirus, Topocuvirus are selected from the group consisting of WDV, MSV, SSV, BYDV, TYDV, BCTV and isolates thereof.

18. Method according to any of the claims 12-17, wherein the gene sequence is selected from the group consisting of C1/AL1/AC1, C2/AL2/AC2, C3/AL3/AC3, C4/AL4/AC4, V1/AR1/AV1, V2/AR2/AV2, BC1/BL1 and BV1/BR1, belonging to the geminiviruses.

19. Method according to claim 18, wherein the C1/AL1/AC1 gene sequence belongs to TYLCSV.

20. Method according to claims 12 and 19, wherein the aminoacid sequence is a truncated protein with respect to the viral wild-type protein.

21. Method according to claims 12-14 and 20 wherein the viral gene sequences made ineffective targets of the virus-induced post-transcriptional gene silencing are the SEQ ID No 8, SEQ ID No 2 and SEQ ID No 4.

22. Method according to claim 21, wherein the truncated proteins are Rep-130 (SEQ ID No 9) or Rep-210 (SEQ ID No 3 and 5).

23. Method according to claim 18, wherein the V1/AR1/AV1 gene sequence belongs to TYLCSV

24. Method according to claims 13 and 23 wherein the viral gene sequence made an ineffective target of the virus-induced post-transcriptional gene silencing is the SEQ ID No 6 encoding for the capsid protein SEQ ID No 7.

25. Method according to anyone of the claims 12-24, wherein the plants, tissues or cells thereof belong to the group consisting of tomato, pepper, tobacco, squash, manioc, sweet potato, cotton, melon, potato, soybean, corn, wheat, sugar cane, bean, beet.